KK 4/4/03

~~claims:~~ What is claimed is:

1. An isolated polynucleotide which codes for the metR and/or metZ genes of coryneform bacteria, selected from the group consisting of

a) polynucleotide which is at least 70% identical to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,

b) polynucleotide which is at least 70% identical to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 3,

c) polynucleotide which codes for a polypeptide that comprises an amino acid sequence which is at least 70% identical to the amino acid sequence of SEQ ID No. 2,

d) polynucleotide which codes for a polypeptide that comprises an amino acid sequence which is at least 70% identical to the amino acid sequence of SEQ ID No. 3,

e) polynucleotide which is complementary to the polynucleotides of a), b), c) or d), and

f) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b), c), d) or e).

2. The polynucleotide as claimed in claim 1, which is capable of replication in coryneform bacteria.

3. The polynucleotide as claimed in claim 1, wherein the polynucleotide is an RNA.

4. The polynucleotide as claimed in claim 2, comprising the nucleic acid sequence as shown in SEQ ID No. 1.

5. A DNA as claimed in claim 2 which is capable of replication, comprising

(i) the nucleotide sequence shown in SEQ ID No. 1, or

(ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or

(iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally

(iv) sense mutations of neutral function in (i).

6. The DNA as claimed in claim 5 which is capable of replication, wherein the hybridization of sequence (iii) is carried out under a stringency corresponding to at most 2x SSC.

7. The polynucleotide sequence as claimed in claim 1, which codes for a polypeptide which comprises the amino acid sequence in SEQ ID No. 2 and/or SEQ ID No. 3.

8. A coryneform bacterium in which the metR gene and/or metZ gene is attenuated.

9. A process for the fermentative preparation of L-amino acids, in particular L-methionine, which comprises carrying out the following steps:

a) fermentation of the coryneform bacteria which produce the desired L-amino acid and in which at least the metR and/or metZ gene or nucleotide sequences which code for them are attenuated.

b) concentration of the L-amino acid in the medium or in the cells of the bacteria, and

c) isolation of the L-amino acid.

10. The process as claimed in claim 9, wherein bacteria in which further genes of the biosynthesis pathway of the

desired L-amino acid are additionally enhanced are employed.

11. The process as claimed in claim 9, wherein bacteria in which the metabolic pathways which reduce the formation of the desired amino acid are at least partly eliminated are employed.

12. The process as claimed in claim 9, wherein the expression of the polynucleotide(s) which code(s) for the metR and/or the metZ gene is reduced or attenuated.

13. The process as claimed in claim 9, wherein the catalytic properties of the polypeptides (enzyme protein) for which the polynucleotides metR and/or metZ code are increased.

14. The process as claimed in claim 9, wherein for the preparation of L-methionine, the coryneform microorganisms have one or more enhanced genes selected from the group consisting of

14.1 the lysC gene which codes for a feed back resistant aspartate kinase,

14.2 the gap gene which codes for glyceroldehyde 3-phosphate dehydrogenase,

14.3 the pyc gene which codes for pyruvate carboxylase,

14.4 the tpi gene which codes for triose phosphate isomerase

14.5 the metA gene which codes for homoserine O-acetyltransferase

14.6 the metB gene which codes for cystathionine gamma-synthase

14.7 the pgk gene which codes for 3-phosphoglycerate kinase

14.8 the aecD gene which codes for cystathionine gamma-lyase

14.9 the glyA gene which codes for serine hydroxymethyltransferase

14.10 the metY gene which codes for O-acetylhomoserine sulfhydrylase.

15. The process as claimed in claim 9, wherein for the preparation of L-methionine, the coryneform microorganisms have one or more attenuated genes selected from the group consisting of

15.1 the thrB gene which codes for homoserine kinase

15.2 the ilvA gene which codes for threonine dehydratase

15.3 the thrC gene which codes for threonine synthase

15.4 the ddh gene which codes for meso-diaminopimelate D-dehydrogenase

15.5 the pck gene which codes for phosphoenol pyruvate carboxykinase

15.6 the pgi gene which codes for glucose 6-phosphate isomerase

15.7 the poxB gene which codes for pyruvate oxidase.

16. The coryneform bacterium which contains a vector which carries a polynucleotide as claimed in claim 1.

17. THe process as claimed in claim 9, wherein microorganisms of the species Corynebacterium glutamicum are employed.

18. The process as claimed in claim 17, wherein the Corynebacterium glutamicum strain ATCC13032deltametRmetZ is employed.

19. A process for preparing L-methionine-containing animal feedstuffs additive comprising

a) culture and fermentation of an L-methionine-producing microorganism in a fermentation medium;

b) removal of water from the L-methionine-containing fermentation broth (concentration);

c) removal of an amount of 0 to 100 wt.% of the biomass formed during the fermentation; and

d) drying of the fermentation broth obtained according to b) and/or c) to obtain the animal feedstuffs additive in the desired powder or granule form.

20. The process as claimed in claim 19, wherein microorganisms are employed in which further genes of the biosynthesis pathway of L-methionine are additionally enhanced.

21. The process as claimed in claim 20, wherein microorganisms are employed in which the metabolic pathways which reduce the formation of L-methionine are at least partly eliminated.

22. The process as claimed in claim 21, wherein the expression of the polynucleotides which code for the metR and/or metZ gene is attenuated.

23. The process as claimed in claim 19, wherein microorganisms of the species Corynebacterium glutamicum are employed.

24. The process as claimed in claim 23, wherein the Corynebacterium glutamicum strain ATCC13032deltametRmetZ is employed.

25. The process as claimed in claim 19, wherein one or more of the following steps are additionally also carried out:

e) addition of one or more organic substances, including L-methionine and/or D-methionine and/or the racemic mixture D,L-methionine, to the products obtained according to b), c) and/or d);

f) addition of auxiliary substances selected from the group consisting of silicas, silicates, stearates, grits and bran to the substances obtained according to b) to e) for stabilization and to increase storability; or

g) conversion of the substances obtained according to b) to f) into a form stable in rumen, by coating with film-forming agents.

26. The process as claimed in claim 19 or 25, wherein a portion of the biomass is removed.

27. The process as claimed in claim 26, wherein essentially 100% of the biomass is removed.

28. The process as claimed in claim 19 or 25, wherein the water content is up to 5 wt.%.

29. The process as claimed in claim 28, wherein the water content is less than 2 wt.%.

30. The process as claimed in claim 25, wherein the film-forming agents are metal carbonates, silicas, silicates, alginates, stearates, starches, gums or cellulose ethers.

31. An animal feedstuffs additive prepared as claimed in claim 19.

32. Then animal feedstuffs additive as claimed in claim 31, which comprises 1 wt.% to 80 wt.% L-methionine, D-methionine, D,L-methionine or a mixture thereof, based on the dry weight of the animal feedstuffs additive.

33. A process for obtaining RNA, cDNA or DNA in order to isolate nucleic acids, or polynucleotides or genes which code for O-succinylhomoserine sulfhydrylase (metZ) and/or

the transcription activator MetR or have a high similarity to the sequence of the O-succinylhomoserine sulfhydrylase (metZ) gene or of the transcription activator MetR, which comprises employing the polynucleotide comprising the polynucleotide sequences as claimed in claim 1, as hybridization probes.